

Claims

1. A method for generating or increasing the resistance of a plant to a plant patho-
gen of the phylum Oomycetes comprising increasing the activity of Rpi-blb2 pro-
tein in the plant or a tissue, organ or cell of a plant or a part thereof.
2. The method of claim 1, wherein said Rpi-blb2 protein is encoded by a poly-
nucleotide comprising a nucleic acid molecule selected from the group consisting
of:
 - (a) nucleic acid molecule encoding at least the mature form of the polypeptide
depicted in SEQ ID NO: 2 or 4;
 - (b) nucleic acid molecule comprising the coding sequence as depicted in SEQ
ID NO: 1 or 3 or 5 or 6 encoding at least the mature form of the polypeptide;
 - (c) nucleic acid molecules the nucleotide sequence of which is degenerate as a
result of the genetic code to a nucleotide sequence of (a) or (b);
 - (d) nucleic acid molecule encoding a polypeptide derived from the polypeptide
encoded by a polynucleotide of (a) to (c) by way of substitution, deletion
and/or addition of one or several amino acids of the amino acid sequence of
the polypeptide encoded by a polynucleotide of (a) to (c);
 - (e) nucleic acid molecule encoding a polypeptide the sequence of which has an
identity of 70% or more to the amino acid sequence of the polypeptide en-
coded by a nucleic acid molecule of (a) or (b);
 - (f) nucleic acid molecule comprising a fragment or a epitope-bearing portion of
a polypeptide encoded by a nucleic acid molecule of any one of (a) to (e);
 - (g) nucleic acid molecule comprising a polynucleotide having a sequence of a
nucleic acid molecule amplified from a nucleic acid library using a primer as
listed in Tab. 3b;
 - (h) nucleic acid molecule encoding a fragment beginning with amino acid: 1,
30, 50, 100, 200, 300, 500, or 1000 and stopping with amino acid 1267,
1000, 500, 300, 200, 50, 30, or 1 of a polypeptide encoded by any one of
(a) to (g);
 - (i) nucleic acid molecule comprising at least 20 nucleotides of a polynucleotide
of any one of (a) or (d);
 - (j) nucleic acid molecule encoding a polypeptide being recognized by a mono-
clonal antibody that have been raised against a polypeptide encoded by a
nucleic acid molecule of any one of (a) to (h);
 - (k) nucleic acid molecule obtainable by screening an appropriate library under
stringent conditions with a probe having the sequence of the nucleic acid
molecule of any one of (a) to (j) or of a fragment thereof of at least 20; and

(l) nucleic acid molecule the complementary strand of which hybridises under stringent conditions with a nucleic acid molecule of any one of (a) or (k);

or the complementary strand of any one of (a) to (l);

5 or expressing a polypeptide encoded by a segment of chromosome or linkage group 6 of *Solanum bulbocastanum* or *Solanum tuberosum* which co-segregates with a marker selected from table 3a or 3b and which mediates resistance to a pathogen of the phylum Oomycetes
and whereby the polynucleotide does not consist of the sequence depicted in
10 Seq. ID NO.: 7 or 9.

3. The method of claim 1 or 2, wherein the activity of at least one further resistance protein is increased.

15 4. The method of any one of claims 1 to 3, wherein activity is increased due to a *de novo*-expression.

5. The method of any one of claims 1 to 4, wherein the endogenous activity of a Rpi-blb2 and/or at least one further resistance protein is increased.

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6. The method of any one of claim 1 to 5, comprising one or more of the following steps

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- a) stabilizing the resistance protein;
 - b) stabilizing the resistance protein encoding mRNA;
 - c) increasing the specific activity of the resistance protein;
 - d) expressing or increasing the expression of a homologous or artificial transcription factor for resistance protein expression;
 - e) stimulate resistance protein activity through exogenous inducing factors;
 - 30 f) expressing a transgenic resistance protein encoding gene; and/or
 - g) increasing the copy number of the resistance protein encoding gene.

7. The method of any one of claims 1 to 6 which results in reduction in the sporulation index of at least 30% after infection with *P. infestans* compared to a wild
35 type.

8. A polynucleotide encoding a Rpi-blb2 protein comprising a nucleic acid molecule selected from the group consisting of:

- 5 (a) nucleic acid molecule encoding at least the mature form of the polypeptide depicted in SEQ ID NO: 2 or 4;
- (b) nucleic acid molecules comprising the coding sequence as depicted in SEQ ID NO: 1 or 3 or 5 or 6 encoding at least the mature form of the polypeptide;
- (c) nucleic acid molecule the nucleotide sequence of which is degenerate as a result of the genetic code to a nucleotide sequence of (a) or (b);
- 10 (d) nucleic acid molecule encoding a polypeptide derived from the polypeptide encoded by a polynucleotide of (a) to (c) by way of substitution, deletion and/or addition of one or several amino acids of the amino acid sequence of the polypeptide encoded by a polynucleotide of (a) to (c);
- 15 (e) nucleic acid molecule encoding a polypeptide the sequence of which has an identity of 70% or more to the amino acid sequence of the polypeptide encoded by a nucleic acid molecule of (a) or (b);
- (f) nucleic acid molecules comprising a fragment or an epitope-bearing portion of a polypeptide encoded by a nucleic acid molecule of any one of (a) to (e);
- (g) nucleic acid molecule comprising a polynucleotide having a sequence of a nucleic acid molecule amplified from a nucleic acid library using the primers as listed in Tab.3b;
- 20 (h) nucleic acid molecule encoding polypeptide fragment beginning with amino acid: 1, 30, 50, 100, 200, 300, 500, or 1000 and stopping with amino acid 1267, 1000, 500, 300, 200, 50, 30, or 1 of a polypeptide encoded by any one of (a) to (g);
- 25 (i) nucleic acid molecule comprising at least 20 nucleotides of a polynucleotide of any one of (a) or (d);
- (j) nucleic acid molecule encoding a polypeptide being recognized by a monoclonal antibodies that have been raised against a polypeptide encoded by a nucleic acid molecule of any one of (a) to (h);
- 30 (k) nucleic acid molecule obtainable by screening an appropriate library under stringent conditions with a probe having the sequence of the nucleic acid molecule of any one of (a) to (j) or of a fragment thereof of at least 20; and
- (l) nucleic acid molecule the complementary strand of which hybridises under stringent conditions with a nucleic acid molecule of any one of (a) or (k);
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or the complementary strand of any one of (a) to (l);

or encoding a polypeptide encoded by a segment of chromosome or of linkage group 6 of *Solanum bulbocastanum* or *Solanum tuberosum* which co-segregates with a marker selected from table 3a or 3b or comprises a replication site or

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hybridisation site for said marker and which mediates resistance to pathogens of the phylum Oomyceta;

and whereby the polynucleotide does not consist of the sequence depicted in Seq. ID NO.: 7 or 9.

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9. The polynucleotide of claim 8 or the method of any one of claims 2 to 7, wherein the marker is E40M58, CT119, or CT216.

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10. The polynucleotide of claim 8 to 9 which is DNA or RNA.

11. A method for making a recombinant vector comprising inserting the polynucleotide of any one of claims 8 to 10 into a vector or inserting said polynucleotide and a further resistance protein.

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12. A vector containing the polynucleotide of any one of claims 8 to 10 or comprising said polynucleotide and a further resistance gene or being produced by the method of claim 11.

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13. The vector of claim 12 or the method of any one of claims 1 to 7 in which a polynucleotide encoding Rpi-blb2 protein or encoding the further resistance protein is operatively linked to expression control sequences and/or is operatively linked to a nucleic acid sequence encoding a transgenic expression regulating signal allowing expression in prokaryotic or eukaryotic host cells.

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14. The vector of claim 12 or 13 or the method of any one of claims 1 to 7 in which the polynucleotide encoding Rpi-blb2 protein or encoding a further resistance protein is operatively linked to expression control sequences of the same species origin as the polynucleotide encoding Rpi-blb2 protein or the further resistance protein.

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15. A method of making a recombinant host cell comprising introducing the vector of any one of claims 12 to 14 or introducing said vector and a vector for expressing a further resistance protein into a host cell.

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16. A host cell produced according to the method of claim 15 or genetically engineered with the polynucleotide of any one of claims 8 to 10 or the vector of any one of claims 12 to 14 or genetically engineered with said vector or polynucleotide and a vector or a polynucleotide for expressing a further resistance protein.

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17. The host cell of claim 16, which is *E. coli*, Baculovirus, Agrobacterium, or a plant cell.
- 5 18. A process for the production of a Rpi-blb2-polypeptide comprising culturing the host cell of claim 16 or 17 and recovering the polypeptide encoded by said polynucleotide and expressed by the host cell from the culture or the host cells.
- 10 19. A polypeptide having the amino acid sequence encoded by a polynucleotide of any one of claims 8 to 10 or obtainable by the process of claim 18 whereby the polypeptide does not consist of the amino acid sequence shown in Seq. ID. No.: 8 and 10..
20. A polypeptide having Rpi-blb2 activity.
- 15 21. An antibody that binds specifically to the polypeptide of claim 19 or 20.
22. An antisense nucleic acid molecule comprising the complementary sequence of the polynucleotide of any one of claims 8 to 10.
- 20 23. A method for the production of a transgenic plant, plant cell or plant tissue or a part thereof comprising the introduction of the polynucleotide of any one of claims 8 to 10 or said polynucleotide and a polynucleotide encoding a further resistance protein, or the vector of any one of claims 12 to 14 into the genome of said plant, plant tissue or plant cell or a part thereof.
- 25 24. A plant cell comprising the polynucleotide of any one of claims 8 to 10, the vector of any one of claims 12 to 14 or obtainable by the method of claim 23.
- 30 25. A transgenic plant or plant tissue or a part thereof comprising the plant cell of claim 24.
- 35 26. A method for producing a plant or a part thereof resistant to a plant pathogen of the phylum Oomycetes comprising the step:
expressing in the plant or a part thereof the polypeptide of claim 19 or 20 and a further resistance protein.
- 40 27. A method for producing a plant or a part thereof with a durable resistance to a *Phytophthora* sp. comprising co-expressing in the plant or a part thereof Rpi-blb and Rpi-blb2 protein or the polypeptide of claim 19 or 20.

28. The transgenic plant or plant tissue of claim 25 or produced according to claim 26 or 27, which upon the presence of the polynucleotide or the vector is resistant to a plant pathogen of the phylum Oomycetes.
- 5 29. Harvestable parts of the transgenic plant or plant tissue of claim 25 comprising the plant cell of claim 24.
30. Propagation material of the transgenic plant or plant tissue of claim 25 comprising the plant cell of claim 24 having an increased Rpi-blb2 activity.
- 10 31. Use of the polynucleotide of any one of claims 8 to 10, the vector of any one of claims 12 to 14, or the polypeptide of claim 19 or 20 for producing a plant or a plant tissue, plant organ, or a plant cell or a part thereof resistant to a plant pathogen of the phylum Oomyceta.
- 15 32. A method for the identification of an compound stimulating resistance to a plant pathogen of the phylum Oomyceta comprising:
- 20 (a) contacting cells which express the polypeptide of claim 19 or 20 or its mRNA with a candidate compound under cell cultivation conditions;
- (b) assaying an increase in expression of said polypeptide or said mRNA;
- (c) comparing the expression level to a standard response made in the absence of said candidate compound; whereby, an increased expression over the standard indicates that the compound is stimulating resistance.
- 25 33. Use of the polynucleotide of any one of claims 8 to 10, the vector of any one of claims 12 to 14, the polypeptide of claim 19 or 20 or the antibody of claim 21, for identifying and/or producing compounds activating or stimulating plant resistance to a plant pathogen of the phylum Oomycetes.
- 30 34. A diagnostic composition, comprising the polynucleotide of any one of claims 8 to 10, the vector of any one of claims 12 to 14, the antibody of claim 21 or the antisense nucleic acid of claim 22 and optionally suitable means for detection.
- 35 35. A kit comprising the polynucleotide of any one of claims 8 or 12, the vector of any one of claims 12 to 14, the host cell of claim 16 or 17, the polypeptide of claim 19 or 20, the antisense nucleic acid of claim 22, the antibody of claim 21, the plant cell of claim 24, the plant or plant tissue of claim 25, the harvestable part of claim 29, or the propagation material of claim 30 and optionally a polynucleotide encoding Rpi-blb, Rpi-blb protein or an antibody against Rpi-blb.
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36. A method for the production of a plant crop protectant providing the polynucleotide of any one of claims 8 to 10, the vector of any one of claims 12 to 14 or the polypeptide of claim 19 or 20 or comprising the steps of the method of claim 32;
5 and formulating the polynucleotide of any one of claims 8 to 10, the vector of of claims 12 or 14 or the polypeptide of claim 19 or 20 or the compound identified in step (c) of claim 32 in a form applicable as agricultural composition.
37. The vector, host cell, plant cell, plant tissue, plant, use, kit or method of any one
10 of claims 1 to 36, wherein the plant pathogen is of the order Pythiales or Peronosperales.
38. The vector, host cell, plant cell, plant tissue, plant, use, kit or method of any
15 one of claims 1 to 37, wherein the plant pathogen is of the species *P. infestans*, *Phytophthora erythroseptica*, *Phytophthora capsici*, *Phytophthora sojae*, *Phytophthora parasitica* var. *nicotianae*, *Bremia lactuca*, *Peronospera tabaci* or *Plasmopara viticola*.
39. The vector, host cell, plant cell, plant tissue, plant, use, kit or method of any one
20 of claims 1 to 38, wherein the resistance protein is characterized by a P-loop and a NBS domain.
40. The vector, host cell, plant cell, plant tissue, plant, use, kit or method of any one
25 of claims 2 to 39, wherein the further resistance gene is a gene encoding Rpi-blb, R1, R-ber, Rpi1, Rpi-blb 3, Rpi-ABPT1, Rpi-mcd, R2, R3a and R3b, R4, R5, R6, R7, R8, R9, R10, R11, Ph-1, Ph-2 and/or Ph-3.
41. The vector, host cell, plant cell, plant tissue, plant, use, kit or method of any one
30 of claims 2 to 40, wherein the further resistance protein is the Rpi-blb protein.
42. The vector, host cell, plant cell, plant tissue, plant, use, kit or method of any
35 one of claims 1 to 41 wherein the plant, plant cell or plant tissue is selected from the group consisting of Menyanthaceae, Solanaceae, Sclerophyllaceae, Duckeodendraceae, Goetzeaceae, Convolvulaceae, Cuscutaceae, Polemoniaceae, and Hydrophyllaceae according to the Systema Naturae 2000, Brands, S.J., Amsterdam or has its origin thereof.
43. The vector, host cell, plant cell, plant tissue, plant, use, kit or method of any
40 one of claims 1 to 42, wherein the polynucleotide, the polypeptide, the plant cell, the host cell, the plant tissue or the plant is derived from the Solanaceae family,

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preferably *S. bulbocastanum*, potato (*S. tuberosum*), tomato (*S. lycopersicum* or *Lycopersicon lycopersicum* (L.) Karsten ex Farwell), petunia, tree tomato (*S. betaceum*), pear melon (*S. muricatum*) or eggplant (*S. melongena*).